A method for measuring coccolith thickness in polarising microscopy that is independent of light intensity

Luc Beaufort, Yves Gally, Baptiste Suchéras-Marx

CEREGE, CNRS/Aix-Marseille University/IRD/College of France/INRA, 13545 Aix-en-Provence, France; beaufort@cerege.fr, gally@cerege.fr, sucheras@cerege.fr

Patrick Ferrand, Julien Duboisset

Institut Fresnel, CNRS/Aix-Marseille University, 13397 Marseille, France; Patrick.ferrand@fresnel.fr, julien.duboisset@fresnel.fr

The birefringence characteristics of coccoliths in polarised microscopy have been used to estimate their mass with linear polarisers (Beaufort, 2005), rotating polarisers (Beaufort et al., 2014) and circular polarisers (Bollmann, 2014; Fuertes et al., 2014). This method is rapid and precise. Camera sensors produce reliable measurements of light that can be converted into thickness. This general methodology has been validated by recent independent measurements made by X-ray tomography at the European Synchrotron Radiation Facility (Beuvier et al., 2019). One of its limitations is that it requires a precise calibration of the microscope brightness. The light intensity, the diaphragm opening, the position of the condenser and the exposure time of the camera have to be strictly identical during the calibration and analysis of the calcite crystal. A slight change in one of these parameters significantly alters the results. Another limitation is that the measured light intensity is not linearly proportional to thickness, but follows a sigmoidal pattern (Beaufort et al., 2014; Bollmann, 2014), making it difficult to estimate thickness precisely at the two ends of the calibration. The use of standard polychromatic light introduces small imprecisions related to the temperature of the light used. There is a theoretical limit to the thickness estimation of around 1.56 μm when using a black and white camera. Here, we propose a new method that solves these problems. The estimations are independent from any calibration or precise tuning of the microscope and light. The calcite thickness results from a simple equation that can be applied to crystals as thick as 2.0 μm. It is based on the alternative use of one left circular polariser and one right circular polariser with a monochromatic light source.

References

Beaufort, L. 2005. Weight estimates of coccoliths using the optical properties (birefringence) of calcite. *Micropaleontology*, **51**(4): 289–298.

Beaufort, L., Barbarin, N. & Gally, Y. 2014. Optical measurements to determine the thickness of calcite crystals and the mass of thin carbonate particles such as coccoliths. *Nature Protocols*, **9**(3): 633–642.

Beuvier, T., Probert, I., Beaufort, L., Suchéras-Marx, B., Chushkin, Y., Zontone, F. & Gibaud, A. 2019. X-ray nanotomography of coccolithophores reveals that coccolith mass and segment number correlate with grid size. *Nature Communications*, **10**(1): 751.

Bollmann, J. 2014. Technical note: Weight approximation of coccoliths using a circular polarizer and interference-colour-derived retardation estimates (the CPR method). *Biogeosciences*, **11**(7): 1899–1910.

Fuertes, M.A., Flores, J.-A. & Sierro, F.J. 2014. The use of circularly polarized light for biometry, identification and estimation of mass of coccoliths. *Marine Micropaleontology*, **113**: 44–55.